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Myostatin: more than just a regulator of muscle mass

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The presence of sufficient skeletal muscle mass is of paramount importance for body function and the myostatin cascade is known to inhibit muscle growth in mammals. In addition, myostatin seems to have an important role in the cross-talk between skeletal muscle and adipose tissue and is involved in insulin sensitivity. In this article we highlight the latest developments related to the myostatin system, emphasizing therapeutic implications for wasting diseases and also the involvement of the system in other organs, in addition to skeletal muscle, such as heart or adipose tissue. Moreover, we highlight the possible role of the myostatin system in the cross-talk between skeletal muscle and adipose tissue, an important aspect that deserves consideration in wasting diseases.

Myostatin: a muscle regulator

Myostatin, also known as growth and differentiation factor-8 (GDF-8), is a member of the transforming growth factor-β (TGF-β) superfamily of secreted growth factors and is a negative regulator of skeletal muscle development [1–3]. During embryogenesis [4], myostatin expression is restricted to developing skeletal muscles, but the protein is still expressed and secreted by skeletal muscles in adulthood [5,6]. Mice and cattle with genetic deficiencies in myostatin exhibit dramatic increases in skeletal muscle mass, therefore supporting the role of myostatin in suppressing muscle growth [7]. Myostatin acts systemically [it is produced in muscle and adipose tissue (to a lesser extend) and released in the circulation] and binds to cell-surface receptors causing muscle loss (Fig. 1). The myostatin protein circulates in the blood in a latent form as a full-length precursor, which is cleaved into an amino-terminal pro-peptide and a carboxy-terminal mature region: the active form of the molecule. Once activated, myostatin has high affinity for the activin IIB receptor (Acvr2b, also known as ActRIIB) and weak affinity for Acvr2a (also known as ActRII and ActRIIA), both of which, similar to other receptors for TGF-β family members, bind multiple ligands [8,9]. In addition to the role of myostatin in skeletal muscle, the peptide has been shown to have a role in adipose and other tissues. This aspect will be analysed in this article.

Myostatin and muscle wasting: the molecular link

Skeletal muscle can both respond and adapt to changing environmental stimuli, leading to a set of metabolic and morphological adaptations that enable it to better meet the energy demands of sustained physical activity. Muscle wasting is associated with catabolic states, such as cancer and infection and is characterized by a decrease in myofibrillar protein content that leads not only to a decrease in skeletal muscle mass, but also in performance [10,11].

In both skeletal muscle and circulation, myostatin is found in inactive complexes of differing composition with other proteins, such as its own pro-peptide, follistatin-like 3 (*Fstl3*, also known as follistatin-related gene (FLRG)), and latent TGF-β binding protein [12–14]. The mechanism of activation of these inactive complexes (or whether all of these complexes are capable of being activated) is unknown. For complexes containing the pro-peptide, activation probably requires proteolysis of the pro-peptide, perhaps by specific target cells [15,16]. Active myostatin binds to its receptor ActRIIB with high affinity and regulates the expression of target genes through a TGF-β signalling pathway (Fig. 1) [6]. Myostatin signalling acts through this receptor on skeletal muscle by setting in motion an intracellular cascade of events. First, there is presumed recruitment of a type I co-receptor. Activin receptor-like

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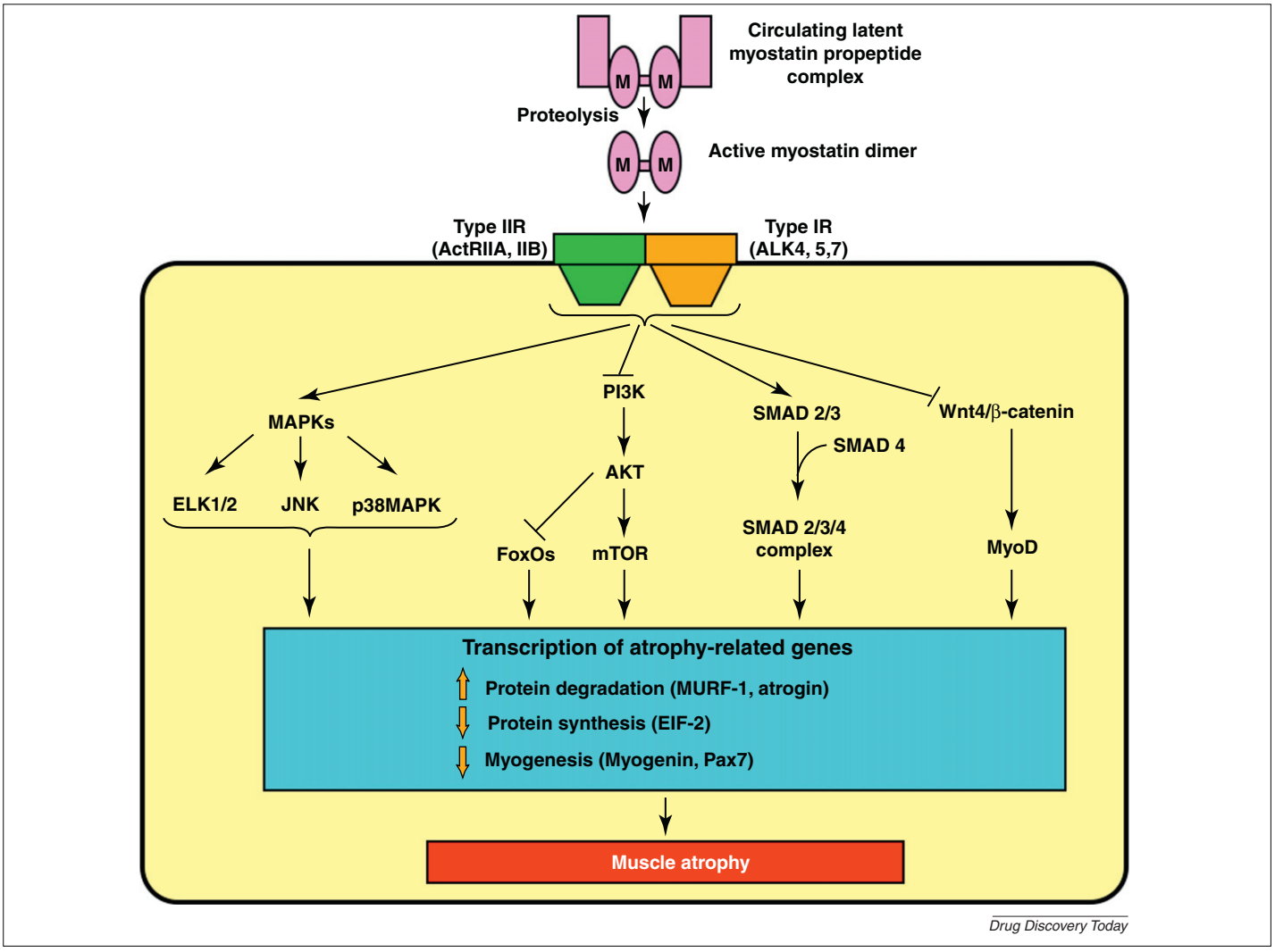

FIGURE 1

Myostatin signalling in skeletal muscle. Myostatin increases protein degradation and decreases protein synthesis by activation of the SMAD complex and by MAPKs and through PI3K/AkT pathway. This results in activation of atrogenic gene expression and inactivation of protein synthesis. In addition, myostatin inhibits the myogenic program, thus resulting in a decrease of myoblast proliferation. *Abbreviations*: JNK: c-Jun N-terminal kinase; MAPKs: mitogen-activated protein kinases. ELK1/2: Ets LiKe gene1; ActRIIA: Activin receptor type IIA; ActRIIB: Activin receptor type IIB; ALK: anaplastic lymphoma receptor tyrosine kinase; PI3K: Phosphatidylinositol 3-kinases; MuRF-1: Muscle RING-finger protein-1; EIF-2: Eukaryotic Initiation Factor 2; Pax7: Paired box protein 7.

kinases 4 and/or 5 (ALK-4, ALK-5) represent candidate co-receptors that are phosphorylated by ActRIIB (Fig. 1). This in turn leads to phosphorylation of TGF-α specific Smads 2 and 3, which forms a complex with Smad 4. The Smad 2/3/4 complex is translocated to the nucleus, to regulate expression of targeted genes such as *MyoD* and other myogenic regulatory factors (Fig. 1) [17]. Smad associates MyoD and inhibits its action [18], therefore suggesting a role in the inhibition of myogenesis. In addition, myostatin can also activate the p38 mitogen-activated protein (MAP)K, Erk1/2, Wnt and c-Jun N-terminal kinase (JNK) signalling pathways (Fig. 1) [6,19–22].

Inhibition of myostatin by injection of neutralizing antibodies or antagonists causes an increase in skeletal muscle mass and therefore myostatin inhibitors have generated great interest as candidates for treatment of muscle wasting diseases [9,23]. By contrast, ectopic expression of myostatin induces atrophy in adult skeletal muscle by decreasing muscle structural genes such as those of myofibrillar proteins (*myosin heavy-chain IIb*, *troponin I* and *desmin*) and myogenic transcription factors (MyoD and myogenin) [24].

Myostatin induces muscle wasting partly by activating the ubiquitin proteolytic system. It has to be pointed out that the muscle loss associated with wasting diseases is linked with an important activation of proteolysis, both in experimental animals [25,26] and humans [27,28]. Among the proteolytic systems involved in protein degradation in muscle during catabolic conditions, the ubiquitin–proteasome system is considered to be the main one [29]. Indeed, the ubiquitin-associated genes *atrogin-1*, *MuRF-1* and 14-kDa ubiquitin-conjugating enzyme E2 (*E214k*) are upregulated following myostatin treatment (Fig. 1) [30]. In fact, myostatin signalling is able to inhibit Akt phosphorylation therefore downregulating the IGF-1/PI3K/AKT hypertrophy pathway (Fig. 1). Myostatin also increases the levels of the active form of the transcription factor FoxO1, enabling increased expression of atrophy-related genes (atrogenes). Although myostatin does not exert its action through Nuclear Factor Kappa B (NF-κB) [30], NF-κB

responsive elements have been found in the promoter of the FLRG, thus indirectly regulating myostatin activity [31].

In addition to muscle protein degradation, myostatin seems to be involved in the control of myogenesis (Fig. 1). Indeed, myostatin can decrease myoblast proliferation in C2C12 cells. It has been suggested that in myoblasts treated with myostatin there is a hypophosphorylation of the Rb protein, suggesting an increase in p21 expression and a decrease in cdk2 protein and activity, thus resulting in an accumulation of hypophosphorylated Rb protein which in turn, leads to the arrest of myoblasts in G1 phase of the cell cycle [32]. Fusion of undifferentiated myoblasts into multinucleated myotubes is a prerequisite for myogenesis. Similar observations have been performed with myotubes, where myostatin inhibits DNA and protein synthesis, therefore affecting cell proliferation [33]. Therefore, from a clinical point of view, the relevance of blocking the myostatin system seems obvious because it would not only ameliorate muscle wasting but also facilitate myogenesis.

Myostatin in different cachexias

Muscle wasting is associated with different pathological states, all of them leading to cachexia. Cachexia is a term originating from the Greek 'Kakos' and 'hexis', meaning 'bad condition'. The cachectic state is observed in many pathological conditions, such as cancer, chronic obstructive pulmonary disease (COPD), sepsis or chronic heart failure (CHF). A 2008 consensus states that cachexia is 'a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass'. The prominent clinical feature of cachexia is weight loss in adults (corrected for fluid retention) or growth failure in children (excluding endocrine disorders). Anorexia, inflammation, insulin resistance and increased muscle protein breakdown are frequently associated with wasting disease. Wasting disease is distinct from starvation, age-related loss of muscle mass, primary depression, malabsorption and hyperthyroidism and is associated with increased morbidity [34]. More recently, Fearon *et al.* have defined cachexia as: 'a multifactorial syndrome defined by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment. Its pathophysiology is characterized by a negative protein and energy balance driven by a variable combination of reduced food intake and abnormal metabolism' [35].

It has been demonstrated that systemic overexpression of myostatin in adult mice could induce profound cachexia with muscle and fat loss analogous to that which is seen in human cancer cachexia syndromes [36].

Cancer

Muscle wasting associated with cancer is a complex process where many mediators seem to be involved, including cytokines [37,38]. Muscle myostatin signalling is enhanced in experimental cancer cachexia. An alteration of the balance myostatin and/or follistatin has been shown in cachectic tumour-bearing rats [39]. The administration of pentoxyfilline resulted in decreased myostatin expression, suggesting a potential influence of TNF-α in the role of myostatin during cancer. In differentiated C2C12 cells, TNF-α induced the expression of myostatin through a p38MAPK-dependent pathway involving NF-κB [40]. Using a myostatin-targeted 2′-*O*-methyl antisense RNA, it has been shown that the administration of the antisense molecules increased muscle weight, both in normal adult mice and in a cancer cachexia mouse model [41]. This effect was specific and is associated with the downregulation of the target gene *myostatin* and the upregulation of the myostatin-modulated myogenic factor *MyoD*. The same authors [42], using a modified RNA oligonucleotide targeting the FoxO1 mRNA in muscle cells, showed that the oligonucleotide could decrease the expression of FoxO1 in cells and *in vivo*, leading to an increase in muscle weight and this being associated with the downregulation of myostatin and upregulation of MyoD. These data clearly support the role of FoxO1 in myostatin activation. Very recently, it has been shown that systemic administration of the activin receptor extracellular domain/Fc fusion protein (ACVR2B-Fc) can inhibit muscle wasting in cachectic mice bearing the colon-26 carcinoma and the Lewis lung carcinoma [43]. Furthermore, the authors also observed that myostatin knockout mice implanted intramuscularly with either Lewis lung carcinoma or B16F20 melanoma cells are more sensitive to tumour-induced cachexia, therefore concluding that host-derived myostatin is not the sole mediator of wasting in cancer [43]. Using a similar approach of pharmacological blockage of the ActRIIB pathway, it has been demonstrated that blocking myostatin signalling not only prevents muscle wasting by reversing muscle loss but also prolongs survival in cachectic tumour-bearing mice [44]. The results of the study suggest a potential control effect of the anti-myostatin treatment because no improvement in inflammatory markers was observed after the treatment [44]. Recent data suggest that alterations in myostatin signalling are also present in human subjects affected by gastric and lung cancer [45].

Chronic heart failure

Cardiac cachexia is characterized by a severe loss of skeletal muscle, weakness, and exercise intolerance, although the cause of these effects remains unknown. It has been recently speculated that the heart functions as an endocrine organ in promoting systemic cachexia by secreting peptide factors, such as myostatin [46]. In their study, cardiomyocyte-specific overexpression of myostatin induced muscle wasting in mice. They also reported that systemic inhibition of myostatin with a blocking antibody in pre-existing heart failure in mice could maintain overall muscle weight at values of sham-operated control mice [46]. Similar observations concerning elevations of myostatin expression in heart have been made using an experimental CHF model [40]. Exercise resulted in a decrease in heart myostatin expression, therefore accounting for the beneficial anti-catabolic effects of exercise in CHF both in experimental models [47] and in human patients [48]. In addition, direct inhibition of myostatin in CHF has shown to positively impact skeletal muscle mass [48].

AIDS

The serum and intramuscular concentrations of myostatin-immunoreactive protein, are increased in HIV-infected men with weight loss compared with healthy subjects and correlate inversely with fat-free mass index [49], this supports the hypothesis that myostatin is an attenuator of skeletal muscle growth in adult men and contributes to muscle wasting in HIV-infected patients.

COPD

An induction of myostatin expression in skeletal muscle of hypoxemic patients with severe COPD has been demonstrated [50]. In addition, using human cell muscle cultures, the same study shows an induction of the myostatin expression in myotubes under hypoxic conditions, suggesting that myostatin can have an essential role in the adaptation of skeletal muscle to hypoxic conditions [50]. Other studies have also shown alterations in myostatin expression in COPD patients both in skeletal muscles [51] and diaphragm [52].

Myostatin in other muscle wasting conditions

Sarcopenia

Age-related skeletal muscle sarcopenia is linked with increases in falls, fractures, and death and therefore has important socioeconomic consequences. The molecular mechanisms controlling age-related muscle loss in humans are not well understood, but are likely to involve multiple signalling pathways. It has been found that both myostatin mRNA and protein levels are significantly elevated in old rats, suggesting an involvement of the protein in age-related muscle wasting [53]. In addition, myostatin-null mice are less prone to sarcopenia and loss of muscle regenerative capacity [54]. Middle-aged men and women had higher serum myostatin levels than young men and women, fat-free mass and muscle mass being inversely correlated with serum myostatin protein concentrations [55]. In addition, antibody-directed myostatin inhibition in aged mice attenuated the decline in muscle mass and function, partly by reducing apoptosis [56].

Duchenne muscular dystrophy and limb-girdle muscular dystrophy 1C

Duchenne muscular dystrophy (DMD) is a recessive disease owing to a mutation in the *dystrophin* gene. Myoblast transplantation permits to introduce the *dystrophin* gene in dystrophic muscle fibres. However, the success of this approach is reduced by the short duration of the regeneration following the transplantation, which reduces the number of hybrid fibres. Myoblast transplantation is enhanced by blocking the myostatin signal with the antagonist follistatin [57]. Blocking myostatin activity by using lentivirus carrying a dominant-negative mutant of ActRIIB resulted in improved myoblast transplantation by increasing cell proliferation and fusion [58]. In addition, several studies with dystrophic mice (*mdx*), a murine model of DMD, have shown that myostatin blockade results in improvement of muscle mass and function [59–62]. Caveolin-3, the muscle-specific isoform of caveolins, has important roles in signal transduction. Dominant-negative mutations of the *caveolin-3* gene cause autosomal dominant limb-girdle muscular dystrophy 1C (LGMD1C) with loss of caveolin-3. However, identification of the precise molecular mechanism leading to muscular atrophy in caveolin-3-deficient muscle has remained elusive. It has been shown that caveolin-3 normally suppresses the myostatin-mediated signal, thereby preventing muscular atrophy, and that hyperactivation of myostatin signalling participates in the pathogenesis of muscular atrophy in a mouse model of LGMD1C [63]. Myostatin inhibition could be a promising therapy for LGMD1C patients. However, in a murine model of LGMD2 C, myostatin blockade improved muscle function but not histopathology [64]. In addition to muscle wasting diseases, the myostatin system seems to be involved in other pathological states.

Insulin resistance and diabetes

In addition to the effects of myostatin on muscle mass and protein accretion, this protein also has an important role in tissue glucose uptake. Blocking myostatin expression results in increased insulin sensitivity associated with increased adiponectin secretion from adipose tissue. In transgenic mice with depressed myostatin function (through muscle-specific expression of the cDNA sequence encoding for the pro-peptide of myostatin), a high (45%) fat diet did not cause glucose intolerance or insulin resistance, as compared with the wild-type animals. In the transgenic animals insulin signalling (as measured by Akt phosphorylation) was significantly elevated [65]. It has been shown that myostatin-deficient mice exhibit reduced insulin resistance through activating the AMP (adenosine monophosphate)-activated protein kinase signalling pathway [66]. This supports a role for myostatin in regulating insulin signalling. Adiponectin is an insulin-sensitizing adipokine known to stimulate fatty acid oxidation in skeletal muscle. An increased secretion of adiponectin might promote energy partition towards skeletal muscles. This suggests a beneficial interaction between muscle and adipose tissue, and might have a role in preventing obesity and insulin resistance. Using a soluble activin receptor type IIB, it has been demonstrated an increase in adiponectin levels together with an increased peripheral glucose uptake [67]. Other pathological states depending on insulin production also seem to be influenced by myostatin. Myostatin has also been associated with type I (insulin-dependent) diabetes mellitus. Muscle myostatin expression levels increase in early stages of type I diabetes in mice. The expression pattern was closely correlated with loss of body weight and atrogin-1 expression. Furthermore, induction of myostatin expression could be attenuated by insulin in type I diabetic mice [68].

The role of myostatin in increasing insulin sensitivity in skeletal muscle is of particular interest in type II diabetic patients.

A putative factor involved in muscle–adipose cross-talk

Traditionally, the regulation of adipose and skeletal muscle masses has always been considered independent. However, different studies suggest that they might be interconnected [69,70]. A potential cross-talk between skeletal muscle and adipose tissue has been proposed and linked with the control of body weight, both fat stores and muscle mass [69]. This represents a completely new way to understand the regulation of body weight control, both total and lean body weight. Adipose tissue and skeletal muscle are reciprocally regulated in two different ways. In normal conditions, signals coming from both tissues might possibly regulate their expansion reciprocally. Therefore, an adequate muscle/adipose mass ratio is maintained. In catabolic conditions different reciprocal signals are released to avoid excessive loss of either adipose or muscle tissue. A direct relationship between skeletal muscle mass or activity and adipose tissue mass has been indirectly suggested in several studies discussing the fact that endurance training, in addition to prevent insulin resistance in humans, can influence both adipose and muscle mass in normal and obese animals and humans. Very recently, Das *et al.* have shown that adipose triglyceride lipase and hormone-sensitive lipase might

have a key role in muscle wasting activation because gene-deficient mice for these enzymes do not show muscle wasting when challenged with a caquectic tumour [71].

The *Mstn* gene is expressed at low levels in adipose tissue and myostatin protein is found in circulation, suggesting that myostatin could have a direct role in regulating adipocyte differentiation or function. *In vitro*, myostatin promotes adipogenesis in the multipotential C3H 10T1/2 mesenchymal cell line [72,73] and inhibits adipogenesis in 3T3L1 preadipocytes, indicating that myostatin actions are different during determination and differentiation steps. *In vivo*, *Mstn* overexpression in adipose tissue results in small immature adipocytes, increased energy expenditure and resistance to diet-induced obesity [74]. Furthermore, the expression of *Mstn*, *Fstl3*, and *Acvr2b* is upregulated in adipocytes from obese mice, suggesting that myostatin signalling might have a role in the response of adipocytes to obesity [74]. Interestingly in the case of follistatin, it seems that the stromal-vascular component is involved in its secretion rather than the adipocytes [75].

It has been shown that increased secretion and expression of myostatin in skeletal muscle from morbid obese women with insulin resistance [76]. Similarly, weight loss reduction results in a decrease in myostatin expression levels [77].

Adipose tissue communicates with skeletal muscle not only through free fatty acids, but also through secretion of various products called adipokines. Adipokines came out as regulators of insulin sensitivity, which are deregulated in obesity. In addition to the well known leptin, adiponectin, interleukin-6 and TNF-α, newer adipokines like retinol-binding protein 4 have been associated with insulin resistance. There is rising evidence that, not only adipose tissue, but also skeletal muscle produces and secretes biologically active proteins or 'myokines' that facilitate metabolic cross-talk between organ systems. Myostatin-deficient mice have a dramatic increase in muscle mass together with a significant reduction in fat depots and a depression of adipogenesis [78]. They also have a resistance to diet-induced and genetic obesity. This suggests that myostatin could have an important role in the cross-talk between skeletal muscle and adipose tissue (Fig. 2) [69]. However, recent studies suggest that myostatin inhibition in muscle, but not in adipose tissue, decreases fat mass and improves insulin sensitivity, suggesting that the decrease in fat mass in myostatin-deficient mice is simply a consequence of metabolic changes in skeletal muscle.

Additional information suggests that myostatin might have an important role in adipose tissue. Zhang *et al.* reported that the absence of myostatin results in an enhanced peripheral tissue fatty acid oxidation and increased thermogenesis therefore increasing fat utilization and reducing storage [79]. On the same lines, Nakatani *et al.* have observed that inhibition of myostatin with a follistatin-derived peptide results in a decrease on adipocyte size, therefore preventing obesity in mice. The treatment also prevented hepatic steatosis and improved glucose tolerance [80]. Li *et al.* suggested that myostatin regulates preadipocite differentiation and lipid metabolism in adipose tissue, this effect being exerted by activation of ERK1/2 [81].

From the data presented, it thus becomes clear that myostatin clearly influences fat mass and this could have positive therapeutic implications in the treatment of obesity and of insulin resistance associated with obesity.

Therapeutic implications and future research

The data presented clearly suggest that, in addition to adipose tissue, skeletal muscle behaves as an endocrine organ; releasing molecules (i.e. myostatin) that have effects on distant targets. This new vision of the adipose–muscular axis could contribute to the future design of new therapeutic approaches for the treatment of diseases related with body weight control, such as obesity or cachexia.

Blocking the myostatin signalling pathway by specific inhibitors might be useful for farming production, treatment of muscle diseases, inhibition of muscle atrophy and last, but not least, as life style drugs in anti-ageing therapies or manipulation of the muscle to fat ratio. Myostatin blockers include myostatin-blocking antibodies, myostatin pro-peptide, follistatin and follistatin-related proteins, soluble myostatin receptors, small interfering RNA and small chemical inhibitors. However, particular care will be necessary for the use of myostatin blocking strategies in clinical practice. For instance, deacetylase inhibitors have been shown to increase muscle cell size by promoting myoblast recruitment and fusion through induction of follistatin [82], Interestingly, in both dystrophin- and α-sarcoglycan-deficient mice, deacetylase inhibitors increase the size of myofibres by inducing follistatin expression in satellite cells [83]. However, the use of these compounds has not been very successful in cancer wasting [43,84].

The influence of exercise on myostatin deserves special attention. From this point of view, following bicycle or swimming exercise in mice, there was a marked increase in plasma follistatin possibly released from the liver [85]. Similarly, post-exercise changes in myostatin and ActRIIB expression in obese insulin-resistant rats have been observed [86]. It has recently been shown that moderate aerobic exercise in insulin-resistant subjects leads to an improvement in insulin sensitivity, which was accompanied by a decrease in circulating myostatin [87]. Similarly, decreases in plasma myostatin following exercise have also been reported in CHF [88] and in stroke patients [89].

The lack of myostatin promotes growth of skeletal muscle, and blockade of its activity has been proposed as a treatment for various muscle-wasting disorders. However, it has been reported that, despite the larger muscle mass in comparison to age-matched wild-types, there was no increase in maximum tetanic strength generation, but when expressed as a function of muscle size (specific strength), muscles of myostatin-deficient mice were weaker than wild-type muscles [90]. In addition, *Mstn* (−/−) muscle contracted and relaxed faster during a single twitch and had a marked increase in the number of type IIb fibres relative to wild-type controls, this change being also accompanied by a significant increase in type IIB fibres containing tubular aggregates. Overall, these results suggest that lack of myostatin compromises strength production in association with loss of oxidative characteristics of the skeletal muscle [90].

Emphasis on myostatin inhibition emerges because treating muscle disorders by most pharmacologic approaches has been disappointing. Glucocorticosteroids, the only beneficial drug treatment for muscular dystrophy, are virtually entirely targeted towards the DMD population. Even in this patient group, the mechanism of benefit is poorly understood, and the evidence that muscle mass is increased is meagre. For genetic muscle diseases,

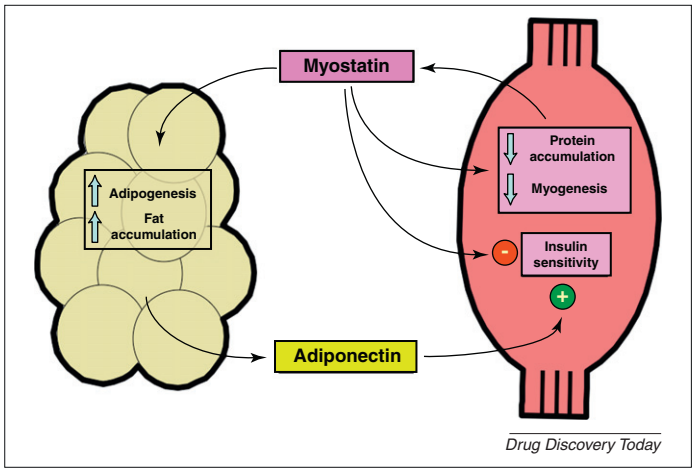

FIGURE 2

Metabolic cross-talk between skeletal muscle and adipose tissue. The effects of myostatin on skeletal muscle are related to both protein accretion and myogenesis. In adipose tissue myostatin increases fat accumulation and increases adipogenesis. Therefore, it acts, as a factor that promotes adipose tissue and decreases skeletal muscle mass. Adiponectin, released by adipose cells, improves insulin sensitivity. It actually acts as a factor which facilitates protein accumulation in skeletal muscle because muscle proteolysis is favoured in insulin resistance conditions. By contrast, myostatin might influence adiponectin synthesis in adipose cells, thus evidencing the opposite roles of these two proteins [95].

gene manipulation strategies are on the horizon, including gene replacement, exon skipping and mutation suppression. Despite enthusiasm, experimental studies suggest that these approaches usually fall short of returning function to normal. Combinational approaches that include partial correction of the underlying defect (i.e. microdystrophin) combined with increasing muscle size and strength appear to offer more. For muscle diseases where correction of the underlying defect might not be an option, increasing muscle size and strength might be opportune for both genetic and acquired muscle diseases where treatment options are limited. Examples include some forms of muscular dystrophy where gene manipulation strategies are not yet applicable [e.g. facioscapulohumeral dystrophy (FSHD)], acquired disorders such as sporadic inclusion body myositis, where pharmacologic treatment failures predominate, or cachectic disorders related to cancer or ageing that might be ideally suited for a muscle-enhancing approach.

Another interesting aspect with possible therapeutic implications is the fact that myostatin can interact with extracellular matrix components, such as fibronectin, laminin, among others [91]. From this point of view, decorin, a small leucine-rich proteoglycan, can sequester myostatin in the extracellular matrix preventing its inhibitory action on myoblast proliferation *in vitro* [92]. Furthermore, overexpression of decorin was able to suppress the activity of myostatin endogenously synthesized in C2C12 myoblasts and attenuated the signalling of exogenous myostatin [92].

Several studies suggest that nutritional approaches, in particular using glutamine, might be useful in partially blocking the myostatin effects. It has been shown that *in vivo* administration of glutamine to experimental animals partly prevents glucocorticosteroid-induced myostatin and muscle atrophy [93]. Similar results have been observed in C2C12 cells cultures where glutamine supplementation effectively prevented TNF-α-induced muscle protein loss and restored normal myostatin levels [94].

Concluding remarks

Altogether, we can conclude that, in addition to gene correction therapy and cell transplantation techniques, multidisciplinary approaches to drug discovery and development offer promising therapeutic strategies for intractable genetic muscular disorders, including muscular dystrophy. Indeed, inhibition of the production and activity of myostatin [41,43,44,83], a potent growth factor that determines skeletal muscle size, is a novel potential strategy for the treatment of muscle-wasting disorders, such as muscular dystrophy, cachexia and sarcopenia.

The fact that myostatin-deficient mice have a dramatic increase in muscle mass together with a significant reduction in fat depots and a depression of adipogenesis, points towards myostatin having a role not only in muscle but also in adipose tissue and this fact could have therapeutic implications in the treatment of obesity.

Future studies should both concentrate on the inhibition of myostatin in relation with disease and involve human subjects owing to the small number of clinical investigations in relation with myostatin.

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References

1 Lee, S.J. and McPherron, A.C. (1999) Myostatin and the control of skeletal muscle mass. *Curr. Opin. Genet. Dev.* 9, 604–607
2 Sharma, M. *et al.* (2001) Myostatin in muscle growth and repair. *Exerc. Sport Sci. Rev.* 29, 155–158
3 Tsuchida, K. (2008) Targeting myostatin for therapies against muscle-wasting disorders. *Curr. Opin. Drug Discov. Devel.* 11, 487–494
4 Amthor, H. *et al.* (2002) The regulation and action of myostatin as a negative regulator of muscle development during avian embryogenesis. *Dev. Biol.* 251, 241–257
5 McPherron, A.C. *et al.* (1997) Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature* 387, 83–90
6 Elkasrawy, M.N. and Hamrick, M.W. (2010) Myostatin (GDF-8) as a key factor linking muscle mass and bone structure. *J. Musculoskelet. Neuronal Interact.* 10, 56–63
7 Lee, S.J. (2007) Sprinting without myostatin: a genetic determinant of athletic prowess. *Trends Genet.* 23, 475–477
8 de Caestecker, M. (2004) The transforming growth factor-beta superfamily of receptors. *Cytokine Growth Factor Rev.* 15, 1–11
9 Guo, T. *et al.* (2009) Myostatin inhibition in muscle, but not adipose tissue, decreases fat mass and improves insulin sensitivity. *PLoS One* 4, E4937
10 Glass, D. and Roubenoff, R. (2010) Recent advances in the biology and therapy of muscle wasting. *Ann. N. Y. Acad. Sci.* 1211, 25–36
11 Puthucheary, Z. *et al.* (2010) Structure to function: muscle failure in critically ill patients. *J. Physiol.* 588, 4641–4648
12 Lee, S.J. (2004) Regulation of muscle mass by myostatin. *Annu. Rev. Cell Dev. Biol.* 20, 61–86
13 Anderson, S.B. *et al.* (2008) Identification of a novel pool of extracellular pro-myostatin in skeletal muscle. *J. Biol. Chem.* 283, 7027–7035

14 Hill, J.J. *et al.* (2002) The myostatin propeptide and the follistatin-related gene are inhibitory binding proteins of myostatin in normal serum. *J. Biol. Chem.* 277, 40735–40741
15 Wolfman, N.M. *et al.* (2003) Activation of latent myostatin by the BMP-1/tolloid family of metalloproteinases. *Proc. Natl Acad. Sci. U. S. A.* 100, 15842–15846
16 Lee, S.J. (2008) Genetic analysis of the role of proteolysis in the activation of latent myostatin. *PLoS One* 3, E1628
17 Rodino-Klapac, L.R. *et al.* (2009) Inhibition of myostatin with emphasis on follistatin as a therapy for muscle disease. *Muscle Nerve* 39, 283–296
18 Langley, B. *et al.* (2002) Myostatin inhibits myoblast differentiation by down-regulating MyoD expression. *J. Biol. Chem.* 277, 49831–49840
19 Allendorph, G.P. *et al.* (2006) Structure of the ternary signaling complex of a TGF-beta superfamily member. *Proc. Natl Acad. Sci. U. S. A.* 103, 7643–7648
20 Steelman, C.A. *et al.* (2006) Transcriptional profiling of myostatin-knockout mice implicates Wnt signaling in postnatal skeletal muscle growth and hypertrophy. *FASEB J.* 20, 580–582
21 Joulia-Ekaza, D. and Cabello, G. (2007) The myostatin gene: physiology and pharmacological relevance. *Curr. Opin. Pharmacol.* 7, 310–315
22 Huang, Z. *et al.* (2007) Regulation of myostatin signaling by c-Jun N-terminal kinase in C2C12 cells. *Cell. Signal.* 19, 2286–2295
23 Whittemore, L.A. *et al.* (2003) Inhibition of myostatin in adult mice increases skeletal muscle mass and strength. *Biochem. Biophys. Res. Commun.* 300, 965–971
24 Durieux, A.C. *et al.* (2007) Ectopic expression of myostatin induces atrophy of adult skeletal muscle by decreasing muscle gene expression. *Endocrinology* 148, 3140–3147
25 Llovera, M. *et al.* (1997) TNF can directly induce the expression of ubiquitin-dependent proteolytic system in rat soleus muscles. *Biochem. Biophys. Res. Commun.* 230, 238–241
26 Busquets, S. *et al.* (2004) Anticachectic effects of formoterol: a drug for potential treatment of muscle wasting. *Cancer Res.* 64, 6725–6731
27 Bossola, M. *et al.* (2001) Increased muscle ubiquitin mRNA levels in gastric cancer patients. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 280, R1518–R1523
28 DeJong, C.H. *et al.* (2005) Systemic inflammation correlates with increased expression of skeletal muscle ubiquitin but not uncoupling proteins in cancer cachexia. *Oncol. Rep.* 14, 257–263
29 Glass, D.J. (2010) Signaling pathways perturbing muscle mass. *Curr. Opin. Clin. Nutr. Metab. Care* 13, 225–229
30 McFarlane, C. *et al.* (2006) Myostatin induces cachexia by activating the ubiquitin proteolytic system through an NF-kappaB-independent, FoxO1-dependent mechanism. *J. Cell. Physiol.* 209, 501–514
31 Bartholin, L. *et al.* (2007) Identification of NF-kappaB responsive elements in follistatin related gene (FLRG) promoter. *Gene* 393, 153–162
32 Thomas, M. *et al.* (2000) Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast proliferation. *J. Biol. Chem.* 275, 40235–40243
33 Taylor, W.E. *et al.* (2001) Myostatin inhibits cell proliferation and protein synthesis in C2C12 muscle cells. *Am. J. Physiol. Endocrinol. Metab.* 280, E221–E228
34 Evans, W.J. *et al.* (2008) Cachexia: a new definition. *Clin. Nutr.* 27, 793–799
35 Fearon, K. *et al.* (2011) Definition and classification of cancer cachexia: an international consensus. *Lancet Oncol.* 12, 489–495
36 Zimmers, T.A. *et al.* (2002) Induction of cachexia in mice by systemically administered myostatin. *Science* 296, 1486–1488
37 Argiles, J.M. *et al.* (2009) The role of cytokines in cancer cachexia. *Curr. Opin. Support. Palliat. Care* 3, 263–268
38 Argiles, J.M. *et al.* (2007) Mechanisms to explain wasting of muscle and fat in cancer cachexia. *Curr. Opin. Support. Palliat. Care* 1, 293–298
39 Costelli, P. *et al.* (2008) Muscle myostatin signalling is enhanced in experimental cancer cachexia. *Eur. J. Clin. Invest.* 38, 531–538
40 Lenk, K. *et al.* (2009) Impact of exercise training on myostatin expression in the myocardium and skeletal muscle in a chronic heart failure model. *Eur. J. Heart Fail.* 11, 342–348
41 Liu, C.M. *et al.* (2008) Myostatin antisense RNA-mediated muscle growth in normal and cancer cachexia mice. *Gene Ther.* 15, 155–160
42 Liu, C.M. *et al.* (2007) Effect of RNA oligonucleotide targeting Foxo-1 on muscle growth in normal and cancer cachexia mice. *Cancer Gene Ther.* 14, 945–952
43 Benny Klimek, M.E. *et al.* (2010) Acute inhibition of myostatin-family proteins preserves skeletal muscle in mouse models of cancer cachexia. *Biochem. Biophys. Res. Commun.* 391, 1548–1554
44 Zhou, X. *et al.* (2010) Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival. *Cell* 142, 531–543
45 Aversa, Z. *et al.* (2011) Changes in myostatin signaling in non-weight-losing cancer patients. *Ann. Surg. Oncol.* PMID: 21519918 (Epub ahead of print)
46 Heineke, J. *et al.* (2010) Genetic deletion of myostatin from the heart prevents skeletal muscle atrophy in heart failure. *Circulation* 121, 419–425
47 Krull, U.J. *et al.* (1988) Towards a fluorescent chemoreceptive lipid membrane-based optode. *Talanta* 35, 129–137
48 Breitbart, A. *et al.* (2011) Myostatin from the heart – local and systemic actions in cardiac failure and muscle wasting. *Am. J. Physiol. Heart Circ. Physiol.* 300, H1973–H1982
49 Gonzalez-Cadavid, N.F. *et al.* (1998) Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. *Proc. Natl. Acad. Sci. U. S. A.* 95, 14938–14943
50 Hayot, M. *et al.* (2011) Myostatin up-regulation is associated with the skeletal muscle response to hypoxic stimuli. *Mol. Cell. Endocrinol.* 332, 38–47
51 Man, W.D-C *et al.* (2010) Quadriceps myostatin expression in COPD. *Eur. Respir. J.* 36, 686–688
52 Testelmans, D. *et al.* (2010) Atrophy and hypertrophy signalling in the diaphragm of patients with COPD. *Eur. Respir. J.* 35, 549–556
53 Leger, B. *et al.* (2008) Human sarcopenia reveals an increase in SOCS-3 and myostatin and a reduced efficiency of Akt phosphorylation. *Rejuvenation Res.* 11, 163B–175B
54 Siriett, V. *et al.* (2006) Prolonged absence of myostatin reduces sarcopenia. *J. Cell. Physiol.* 209, 866–873
55 Yarasheski, K.E. *et al.* (2002) Serum myostatin-immunoreactive protein is increased in 60–92 year old women and men with muscle wasting. *J. Nutr. Health Aging* 6, 343–348
56 Murphy, K.T. *et al.* (2010) Antibody-directed myostatin inhibition in 21-mo-old mice reveals novel roles for myostatin signaling in skeletal muscle structure and function. *FASEB J.* 24, 4433–4442
57 Benabdallah, B.F. *et al.* (2008) Inhibiting myostatin with follistatin improves the success of myoblast transplantation in dystrophic mice. *Cell Transplant.* 17, 337–350
58 Fakhfakh, R. *et al.* (2011) Blocking the myostatin signal with a dominant negative receptor improves the success of human myoblast transplantation in dystrophic mice. *Mol. Ther.* 19, 204–210
59 Bogdanovich, S. *et al.* (2002) Functional improvement of dystrophic muscle by myostatin blockade. *Nature* 420, 418–421
60 Qiao, C. *et al.* (2008) Myostatin propeptide gene delivery by adeno-associated virus serotype 8 vectors enhances muscle growth and ameliorates dystrophic phenotypes in mdx mice. *Hum. Gene Ther.* 19, 241–254
61 Morine, K.J. *et al.* (2010) Systemic myostatin inhibition via liver-targeted gene transfer in normal and dystrophic mice. *PLoS One* 5, E9176
62 Dumonceaux, J. *et al.* (2010) Combination of myostatin pathway interference and dystrophin rescue enhances tetanic and specific force in dystrophic mdx mice. *Mol. Ther.* 18, 881–887
63 Ohsawa, Y. *et al.* (2006) Muscular atrophy of caveolin-3-deficient mice is rescued by myostatin inhibition. *J. Clin. Invest.* 116, 2924–2934
64 Bogdanovich, S. *et al.* (2008) Myostatin blockade improves function but not histopathology in a murine model of limb-girdle muscular dystrophy 2C. *Muscle Nerve* 37, 308–316
65 Zhao, B. *et al.* (2005) Transgenic expression of myostatin propeptide prevents diet-induced obesity and insulin resistance. *Biochem. Biophys. Res. Commun.* 337, 248–255
66 Zhang, C. *et al.* (2011) Myostatin-deficient mice exhibit reduced insulin resistance through activating the AMP-activated protein kinase signalling pathway. *Diabetologia* 54, 1491–1501
67 Akpan, I. *et al.* (2009) The effects of a soluble activin type IIB receptor on obesity and insulin sensitivity. *Int. J. Obes. (Lond.)* 33, 1265–1273
68 Chen, Y. *et al.* (2009) Upregulation of myostatin gene expression in streptozotocin-induced type 1 diabetes mice is attenuated by insulin. *Biochem. Biophys. Res. Commun.* 388, 112–116
69 Argiles, J.M. *et al.* (2005) Cross-talk between skeletal muscle and adipose tissue: a link with obesity? *Med. Res. Rev.* 25, 49–65
70 Trayhurn, P. *et al.* (2011) Secreted proteins from adipose tissue and skeletal muscle – adipokines, myokines and adipose/muscle cross-talk. *Arch. Physiol. Biochem.* 117, 47–56
71 Das, S.K. *et al.* (2011) Adipose triglyceride lipase contributes to cancer-associated cachexia. *Science* 333, 233–238
72 Artaza, J.N. *et al.* (2005) Myostatin inhibits myogenesis and promotes adipogenesis in C3H 10T(1/2) mesenchymal multipotent cells. *Endocrinology* 146, 3547–3557
73 Feldman, B.J. *et al.* (2006) Myostatin modulates adipogenesis to generate adipocytes with favorable metabolic effects. *Proc. Natl. Acad. Sci. U. S. A.* 103, 15675–15680
74 Allen, D.L. *et al.* (2008) Myostatin, activin receptor IIb, and follistatin-like-3 gene expression are altered in adipose tissue and skeletal muscle of obese mice. *Am. J. Physiol. Endocrinol. Metab.* 294, E918–E927
75 Flanagan, J.N. *et al.* (2009) Role of follistatin in promoting adipogenesis in women. *J. Clin. Endocrinol. Metab.* 94, 3003–3009

76 Hittel, D.S. *et al.* (2009) Increased secretion and expression of myostatin in skeletal muscle from extremely obese women. *Diabetes* 58, 30–38
77 Milan, G. *et al.* (2004) Changes in muscle myostatin expression in obese subjects after weight loss. *J. Clin. Endocrinol. Metab.* 89, 2724–2727
78 McPherron, A.C. and Lee, S.J. (2002) Suppression of body fat accumulation in myostatin-deficient mice. *J. Clin. Invest.* 109, 595–601
79 Zhang, C. *et al.* (2012) Inhibition of myostatin protects against diet-induced obesity by enhancing fatty acid oxidation and promoting a brown adipose phenotype in mice. *Diabetologia* 55, 183–193
80 Nakatani, M. *et al.* (2011) Follistatin-derived peptide expression in muscle decreases adipose tissue mass and prevents hepatic steatosis. *Am. J. Physiol. Endocrinol. Metab.* 300, E543–E553
81 Li, F. *et al.* (2011) Myostatin regulates preadipocyte differentiation and lipid metabolism of adipocyte via ERK1/2. *Cell Biol. Int.* 35, 1141–1146
82 Iezzi, S. *et al.* (2004) Deacetylase inhibitors increase muscle cell size by promoting myoblast recruitment and fusion through induction of follistatin. *Dev. Cell* 6, 673–684
83 Minetti, G.C. *et al.* (2006) Functional and morphological recovery of dystrophic muscles in mice treated with deacetylase inhibitors. *Nat. Med.* 12, 1147–1150
84 Bonetto, A. *et al.* (2009) Deacetylase inhibitors modulate the myostatin/follistatin axis without improving cachexia in tumor-bearing mice. *Curr. Cancer Drug Targets* 9, 608–616
85 Hansen, J. *et al.* (2011) Exercise induces a marked increase in plasma follistatin: evidence that follistatin is a contraction-induced hepatokine. *Endocrinology* 152, 164–171
86 Bueno, P.G. *et al.* (2011) Post-exercise changes in myostatin and actRIIB expression in obese insulin-resistant rats. *Mol. Cell. Endocrinol.* 339, 159–164
87 Hittel, D.S. *et al.* (2010) Myostatin decreases with aerobic exercise and associates with insulin resistance. *Med. Sci. Sports Exerc.* 42, 2023–2029
88 Lenk, K. *et al.* (2011) Exercise training leads to a reduction of elevated myostatin levels in patients with chronic heart failure. *Eur. J. Cardiovasc. Prev. Rehabil.* PMID: 21450574 (Epub ahead of print)
89 Ryan, A.S. *et al.* (2011) Skeletal muscle hypertrophy and muscle myostatin reduction after resistive training in stroke survivors. *Stroke* 42, 416–420
90 Amthor, H. *et al.* (2007) Lack of myostatin results in excessive muscle growth but impaired force generation. *Proc. Natl. Acad. Sci. U. S. A.* 104, 1835–1840
91 Miura, T. *et al.* (2010) Interaction between myostatin and extracellular matrix components. *Anim. Sci. J.* 81, 102–107
92 Kishioka, Y. *et al.* (2008) Decorin enhances the proliferation and differentiation of myogenic cells through suppressing myostatin activity. *J. Cell. Physiol.* 215, 856–867
93 Salehian, B. *et al.* (2006) The effect of glutamine on prevention of glucocorticoid-induced skeletal muscle atrophy is associated with myostatin suppression. *Metabolism* 55, 1239–1247
94 Bonetto, A. *et al.* (2011) Glutamine prevents myostatin hyperexpression and protein hypercatabolism induced in C2C12 myotubes by tumor necrosis factor-alpha. *Amino Acids* 40, 585–594
95 Suzuki, S.T. *et al.* (2008) Enhanced muscle by myostatin propeptide increases adipose tissue adiponectin, PPAR-alpha, and PPAR-gamma expressions. *Biochem. Biophys. Res. Commun.* 369, 767–773